Radical Enzymes

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Hydrogen Bonds Guide the Short-Lived 5′-Deoxyadenosyl Radical to the Place of Action**

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Coenzyme B_{12} (5′-deoxyadenosylcobalamin) is a cofactor for two types of enzymes: mutases and eliminases. Mutases catalyze reversible rearrangements of carbon skeletons, for example, (*S*)-glutamate to (2*S*,3*S*)-3-methylaspartate, and the migration of amino groups to adjacent carbons. The eliminases are responsible for the irreversible elimination of hydroxy groups from vicinal diols or amino groups from 1,2-aminoalcohols, for example, the dehydration of glycerol to 3-hydroxypropanal. These radical enzymes share a common mechanism, in which a hydrogen atom and a substituent swap places on neighboring sp^3-hybridized carbon atoms.[1] An intensively studied example is methylmalonyl-CoA mutase (CoA = coenzyme A), which occurs in animal mitochondria and bacteria, and catalyzes the interconversion of (*R*)-methylmalonyl-CoA with succinyl-CoA (Scheme 1). The binding of (*R*)-methylmalonyl-CoA or succinyl-CoA to the enzyme induces the homolytic cleavage of the cobalt–carbon σ-bond of the coenzyme generating cob(II)alamin and the 5′-deoxyadenosyl radical that initiates catalysis. This cleavage occurs 10^{12}-fold faster with the enzyme than in aqueous solutions of the unbound coenzyme.[2] The 5′-deoxyadenosyl radical abstracts a hydrogen atom from the methyl group of methylmalonyl-CoA to afford 5′-deoxyadenosine and the methylenemalonyl-CoA radical, which rearranges to the 3-succinyl-CoA radical probably via a cyclic intermediate (Scheme 1 B).[3] Subsequent hydrogen transfer from the methyl group of 5′-deoxyadenosine to the succinyl radical yields succinyl-CoA and regenerates the 5′-deoxyadenosyl radical, which recombines with cob(II)alamin.[1] Evidence for this mechanism was provided by EPR studies, which showed a hybrid triplet spin system arising from exchange and dipolar couplings between an organic radical and the low-spin Co^{2+} center of cob(II)alamin, which are separated by about 660 pm (6.6 Å). With the aid of ^{13}C-labeled methylmalonyl-CoA samples, the 3-succinyl-CoA radical, which is stabilized by the carboxylate group, was identified, but the two methylene radicals, in particular the 5′-deoxyadenosyl radical, remained elusive.[4] Similar experiments were performed with glutamate mutase.[5]

The 5′-deoxyadenosyl radical is a highly reactive species without resonance stabilization and it possesses an adenine moiety that can attach the radical to the protein. Owing to rotation about the C–N bond linking adenine and ribose, this radical can abstract a hydrogen atom by moving towards the substrate, which is bound 870 pm from the cobalt in the eliminases.[6] In contrast, in the mutases pseudorotation of the ribose moiety from the C2′-*endo* to the C3′-*endo* conformation at a fixed C–N torsion angle restricts this distance to 660 pm.[7] Owing to its extremely short lifetime of roughly 2 ns, the 5′-deoxyadenosyl radical has never been observed by EPR during enzyme turnover. The lifetime of the intermediate adenosyl radical was prolonged by substituting 5′-deoxy-3′,4′-anhydroadenosylcobalamin as the cofactor for coenzyme B_{12}-dependent diol dehydratase.[8] EPR spectra showed the formation of 5′-deoxy-3′,4′-anhydroadenosyl radical, but turnover was 5000-fold slower, because this resonance-stabilized allylic radical is much less reactive than the 5′-deoxyadenosyl radical.

The elusiveness of the 5′-deoxyadenosyl radical prompted several researchers to propose a concerted mechanism for the mutases, in which fission of the Co–C bond and hydrogen abstraction from the substrate occur simultaneously (Scheme 1). However, the 660 pm distance between the Co atom and substrate mentioned above disfavors this scenario.[7] The observed weak coupling between Co^{2+} of cob(II)alamin and the substrate-derived organic radical ("*There are two radicals talking to each other*" Brian Hoffman, Northwestern University, USA), which would be stronger between Co^{2+} and the 5′-deoxyadenosyl radical on its way to the substrate through pseudorotation of the ribose moiety, was taken as evidence for cob(II)alamin acting as a "conductor" rather than as a mere "spectator".[9]

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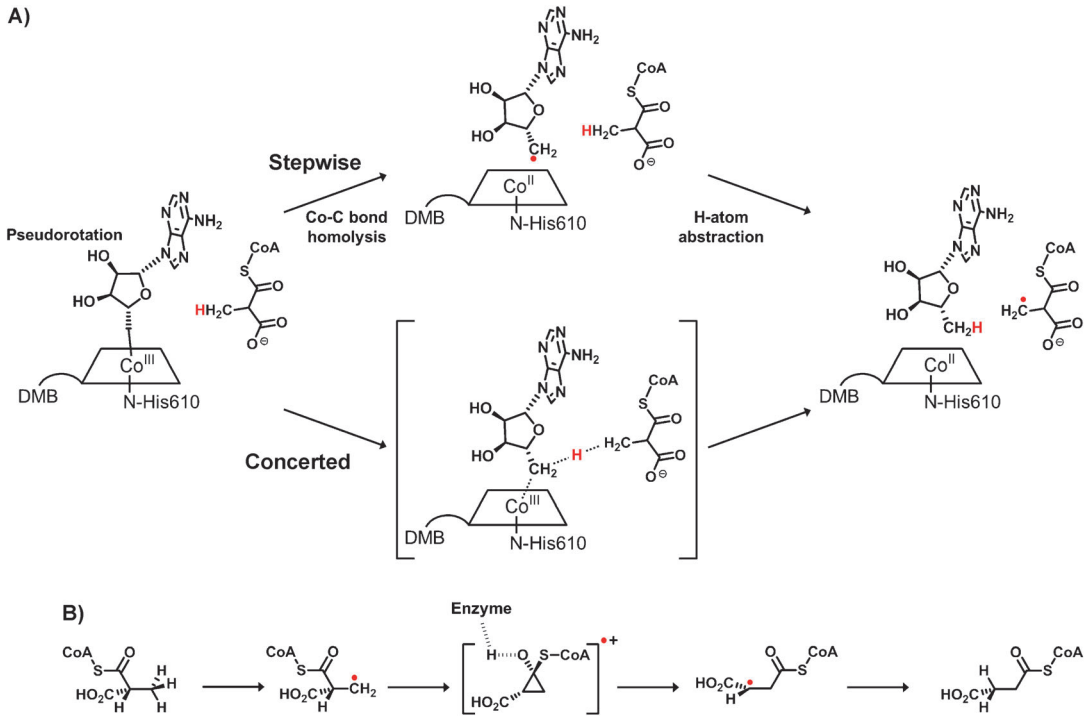

Scheme 1. Mechanism of methylmalonyl-CoA mutase. A) Stepwise vs. concerted hydrogen-atom abstraction (modified from Ref. [13]). B) Thioester migration via a cyclic intermediate (stabilized by partial protonation; modified from Ref. [3]). DMB = 5,6-dimethylbenzimidazole.

Gas-phase calculations led to the postulate of a strong hydrogen bond ($\Delta G \approx -30\ kJ\,mol^{-1}$) between C19-H of the coenzyme and the ribose 3′-O position of the 5′-deoxyadenosyl radical, which arises after Co–C bond homolysis and pseudorotation of the ribose moiety, and guides the radical to the substrate.[10] However, the lack of exchange of C19-H with D_2O and the ability of 3′,5′-dideoxyadenosylcobalamin to act as a coenzyme for glutamate mutase with only 10-fold lower k_{cat}/K_m compared to coenzyme B_{12} indicated a much weaker interaction of $\Delta G \geq -7\ kJ\,mol^{-1}$.[11] In contrast, the 2′-OH group of coenzyme B_{12} appeared to be important for catalysis because its deletion resulted in almost complete loss of activity with glutamate mutase[11] as well as with methylmalonyl-CoA mutase.[12]

Bucher et al. have used molecular dynamics simulations in a mixed quantum mechanics/molecular mechanics framework in a highly detailed study of methylmalonyl-CoA mutase.[13] These calculations included the interactions of the amino acid side chains above and below the plane of the coenzyme, especially focusing on how the protein enhances Co–C5′ bond homolysis and guides the emerging radical to the substrate. An important result was the calculation of a free energy profile for the homolysis of the Co–C5′ bond and hydrogen abstraction from the substrate along the Co–C5′ and C5′–H coordinates. Starting at 205 pm, the profile displayed two maxima, one at 360 pm corresponding to the activation energy of Co–C5′ bond cleavage ($64\ kJ\,mol^{-1}$), and the other at 600 pm relating to the activation energy for hydrogen abstraction ($82\ kJ\,mol^{-1}$). A shallow minimum at 400–450 pm ($56\ kJ\,mol^{-1}$) indicated that the 5′-deoxyadenosyl radical is a discrete species. Although the profile suggests that the most favorable reaction of the radical is recombination with cob(II)alamin rather than hydrogen abstraction, further calculations revealed a developing hydrogen bond between the 2′-OH group of the radical and glutamate 370 of the enzyme (Figure 1). Thus, the radical is drawn to the substrate

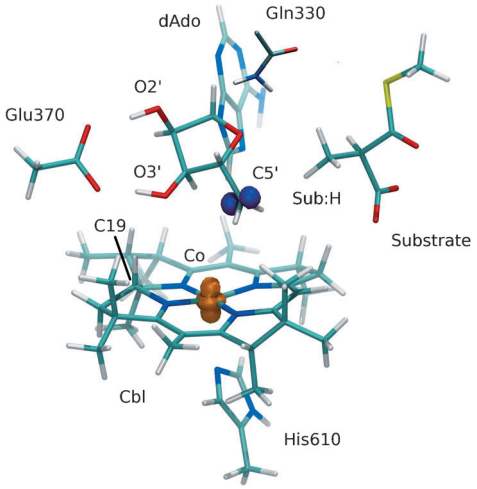

Figure 1. Addition of (*R*)-methylmalonyl-CoA [in the calculations and in the figure simplified as (*R*)-methylmalonyl-methylthiol] to methylmalonyl-CoA mutase with bound coenzyme B_{12} (Cbl) causes homolysis of the Co–C bond and the approach of the 5′-deoxyadenosyl radical (dAdo) to the methyl group of the substrate by pseudorotation of the ribose moiety aided by the formation of hydrogen bonds between glutamate 370 and 2′-OH as well as between C19-H and 3′-O. (Figure 3a from Ref. [13]). Sub:H = Hydrogen atom of the substrate to be abstracted by dAdo.

by pseudorotation of the flexible ribose moiety. In contrast the hydrogen bond between 3′-OH and glutamate 370 remains almost unchanged during the movement of the 5′-deoxyadenosyl radical from the Co atom to the vicinity of the substrate, which supports our experimental results with glutamate mutase.[11] A further push towards the substrate comes from the developing hydrogen bond between C19-H and 3′-OH, although the authors confess "*the magnitude of this stabilization appears to be somewhat less in the enzymatic environment than in the gas phase*".[13] In addition, the activation energy of the homolysis is lowered by elongation of the Co–N bond to the axial histidine 610 from 230 to 400 pm, assisted by formation of a hydrogen bond from the neighboring aspartate 608 (Figure 1). After the Co···C5′ distance of approximately 425 pm is reached, when the 5′-deoxyadenosyl radical has formed, the histidine returns to its original position.[13] The sequence of events described is beautifully illustrated in a video that accompanies the paper[13] and perhaps for mechanistic publications this is "*The Shape of Things to Come*" (H. G. Wells, 1933).

The authors' proposed mechanism, with several steps and conformational changes, recalls János Rétey's idea of "negative catalysis", by which the enzyme maintains highly reactive species in an environment that prevents possible side reactions and guides them from their site of genesis to their place of action.[14] The authors thus challenge the concept of stabilizing the transition state as the central paradigm of enzyme catalysis. The work of Bucher et al. also demonstrates the importance of conformational changes during catalysis, which most likely happen with every enzyme. Finally, it has to be emphasized that this work would not have been possible without information on the crystal structure of methylmalonyl-CoA mutase with bound coenzyme and substrate,[15] as well as detailed kinetic analyses.[16]

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[1] W. Buckel, B. T. Golding in *Handbook of Radicals in Chemistry, Biology and Materials* (Eds.: C. Chatgilialoglu, A. Studer), Wiley, Chichester, UK, **2012**, pp. 1501 – 1546.
[2] B. P. Hay, R. G. Finke, *J. Am. Chem. Soc.* **1987**, *109*, 8012 – 8018.
[3] D. M. Smith, B. T. Golding, L. Radom, *J. Am. Chem. Soc.* **1999**, *121*, 9388 – 9399.
[4] S. O. Mansoorabadi, R. Padmakumar, N. Fazliddinova, M. Vlasie, R. Banerjee, G. H. Reed, *Biochemistry* **2005**, *44*, 3153 – 3158.
[5] H. Bothe, D. J. Darley, S. P. Albracht, G. J. Gerfen, B. T. Golding, W. Buckel, *Biochemistry* **1998**, *37*, 4105 – 4113.
[6] G. Bender, R. R. Poyner, G. H. Reed, *Biochemistry* **2008**, *47*, 11360 – 11366.
[7] K. Gruber, R. Reitzer, C. Kratky, *Angew. Chem.* **2001**, *113*, 3481 – 3484; *Angew. Chem. Int. Ed.* **2001**, *40*, 3377 – 3380.
[8] O. T. Magnusson, G. H. Reed, P. A. Frey, *Biochemistry* **2001**, *40*, 7773 – 7782.
[9] W. Buckel, C. Kratky, B. T. Golding, *Chem. Eur. J.* **2005**, *12*, 352 – 362.
[10] B. Durbeej, G. M. Sandala, D. Bucher, D. M. Smith, L. Radom, *Chem. Eur. J.* **2009**, *15*, 8578 – 8585.
[11] P. Friedrich, F. Baisch, R. Harrington, F. E. Lyatuu, K. Zhou, F. Zelder, W. McFarlane, W. Buckel, B. T. Golding, *Chem. Eur. J.* **2012**, DOI: 10.1002/chem.201201840.
[12] A. M. Calafat, S. Taoka, J. M. Puckett, Jr., C. Semerad, H. Yan, L. Luo, H. Chen, R. Banerjee, L. G. Marzilli, *Biochemistry* **1995**, *34*, 14125 – 14130.
[13] D. Bucher, G. M. Sandala, B. Durbeej, L. Radom, D. M. Smith, *J. Am. Chem. Soc.* **2012**, *134*, 1591 – 1599.
[14] J. Rétey, *Angew. Chem.* **1990**, *102*, 373 – 379; *Angew. Chem. Int. Ed. Engl.* **1990**, *29*, 355 – 361.
[15] F. Mancia, G. A. Smith, P. R. Evans, *Biochemistry* **1999**, *38*, 7999 – 8005.
[16] A. Dybala-Defratyka, P. Paneth, R. Banerjee, D. G. Truhlar, *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 10774 – 10779.

